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Applicant: Ault-Riche et al.
DKT. No. 25885-1751
Priority claimed to 60/219,183
For:COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Sorting by pools

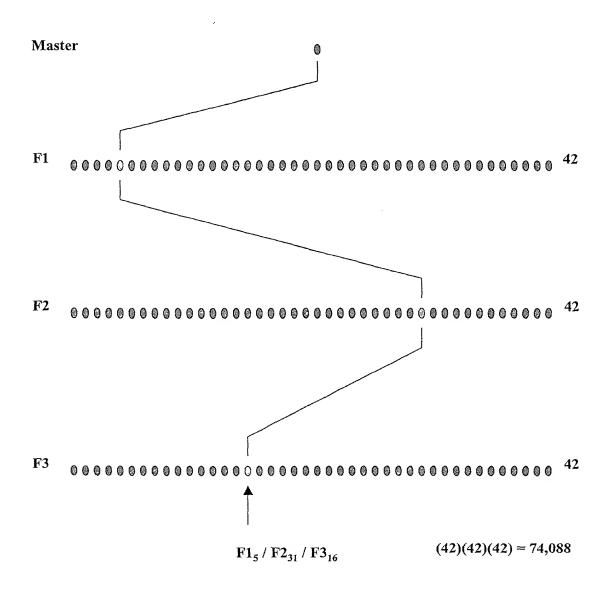


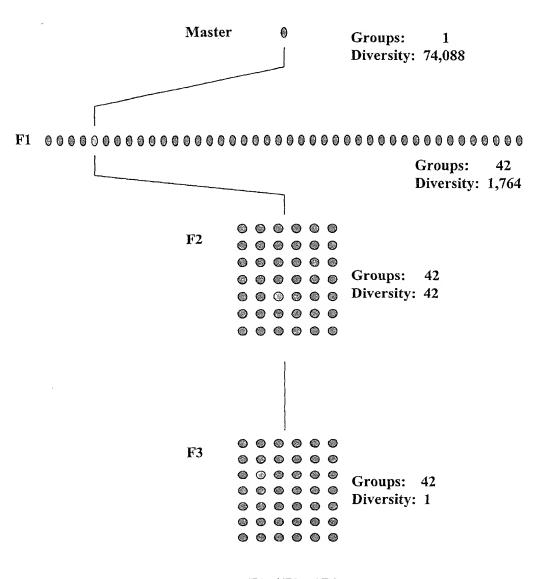
FIGURE 1

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Sorting by pools: Decreasing pool diversities

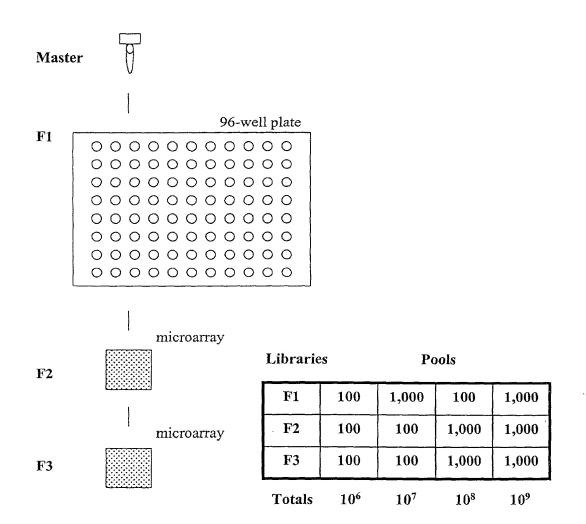


F1₅ / F2₃₁ / F3₁₆

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Sorting by pools: Screening large diversity libraries



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Searching a mutation library

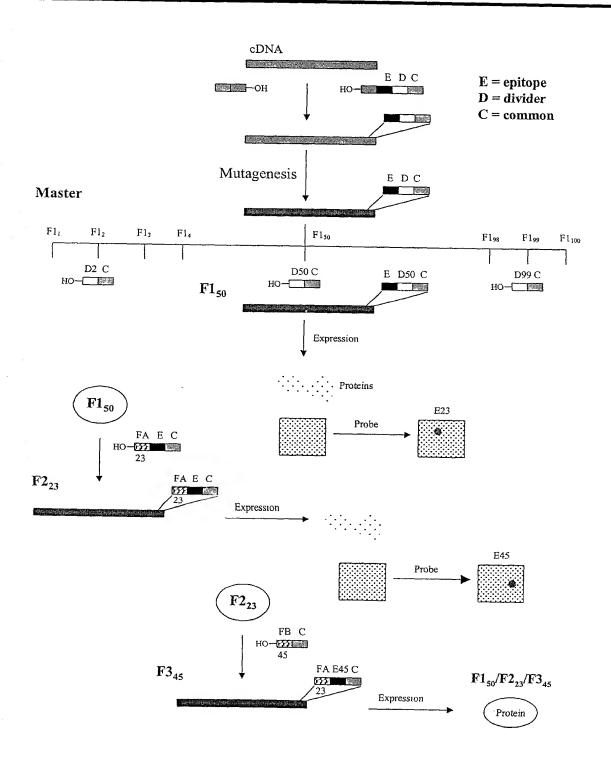
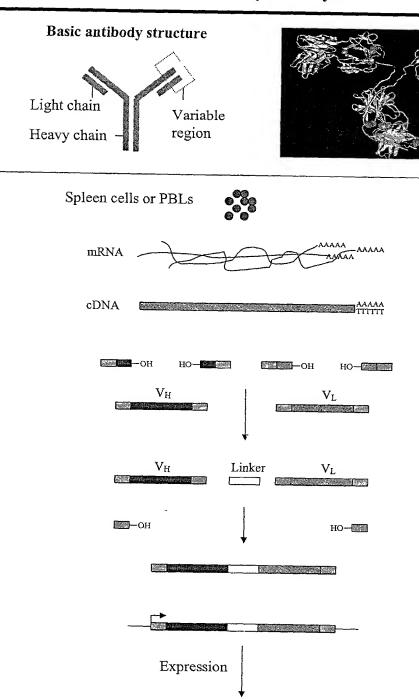


FIGURE 4

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Making a recombinant antibody library



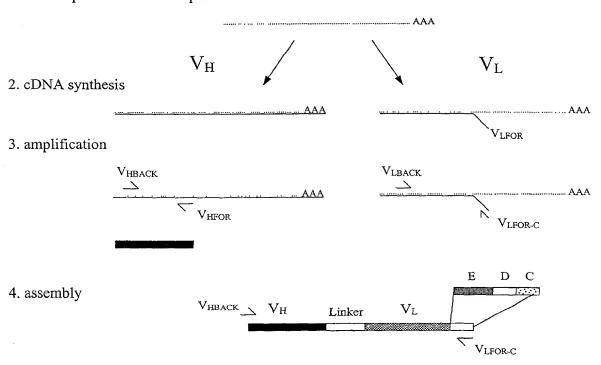
Antibodies

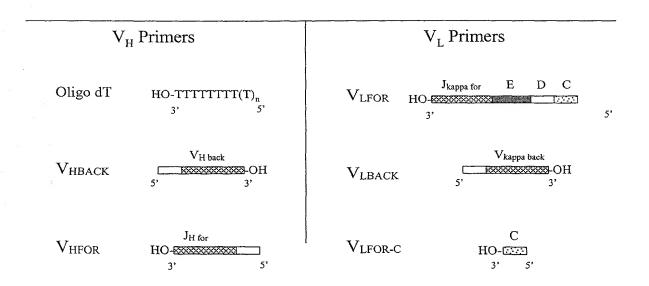
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Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs

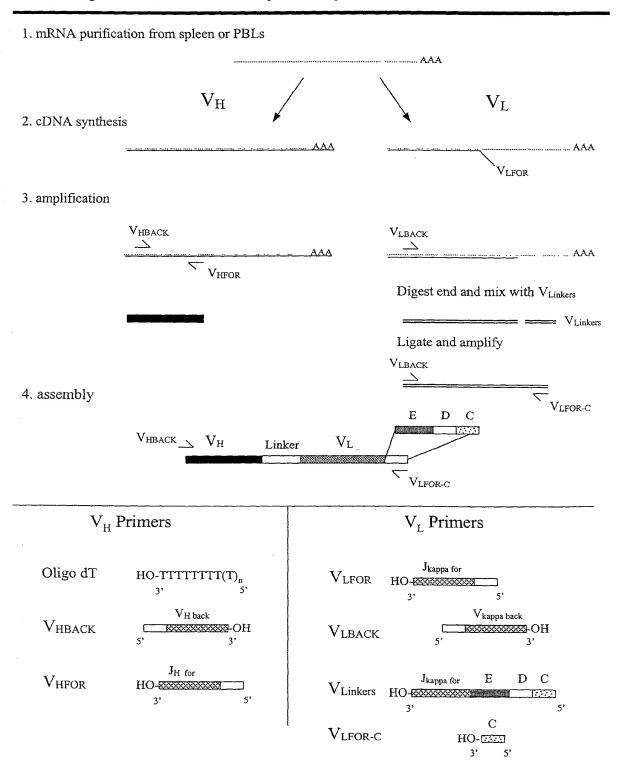




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Creating the master antibody library: Linker addition



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Searching a recombinant antibody library

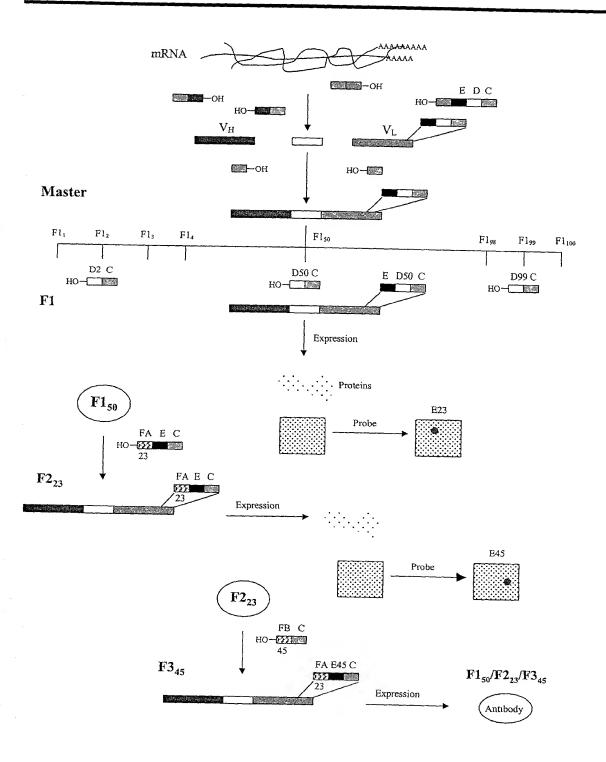


FIGURE 8

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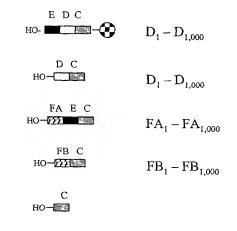
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FOR:COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING

Physical elements to include in the kits and combinations

- Anti-tag Arrays™
- Primer sets



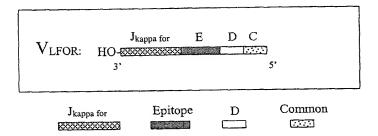
- Readers
- Software

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For:COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING

Making the V_{LFOR} primers: Solid phase synthesis



1. Synthesize oligo on solid support

2. Add aminolink prior to cleavage

3. Couple to tosyl activated magnetic beads

4. Extend by hybridizing with DNA patch and ligating

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Making the $V_{LFOR}\ primers\colon Overlapping\ hybridization$

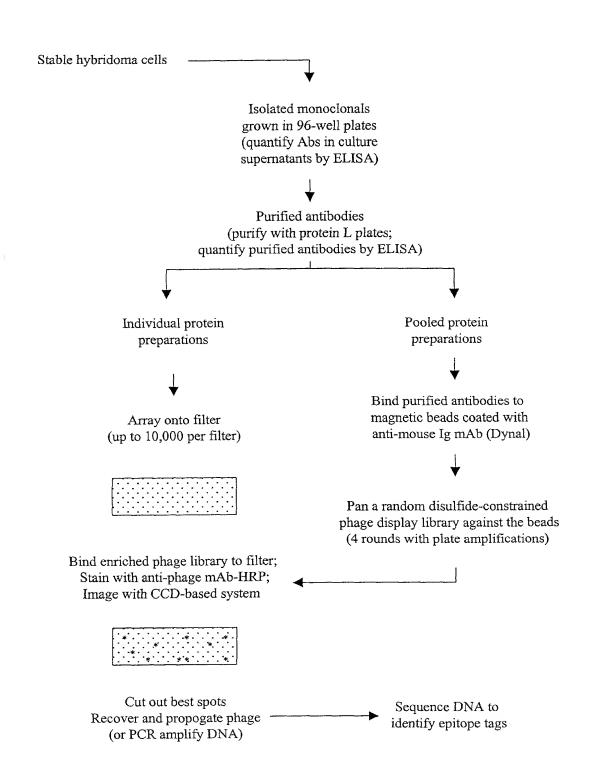
	VLFOR: HO-************************************
	J _{kappa for} Epitope D Common
•	Synthesize 4,028 different oligos: (26 for J _{kappa for} ; 2,000 for Epitope; 2,000 for D; 2 for Common)
2.	Assemble oligos for + and - strands of the different regions
	НО
3.	Ligase the assembled oligos
	но
4.	1st strand synthesis with biotinylated primer
	но он
•	2 nd strand synthesis with non-biotinylated primer
	но он
6.	Bind to avidin coated magnetic beads and then denature
	У*ОН
	НО
7.	Purify non-biotinylated ssDNA J_{kappa for} Epitope D Common HO

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Applicant: Ault-Riche et al.

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Building the collection of antibody/tag pairs: Hybridoma screening



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For:COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING

FIGURE 13A

TABLE 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH back primers (sense)

HuVHlaBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5' GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Human'IH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC OOT OAC CAT TOT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVklaBACK	51-GAC ATC CAG ATG ACC CAG TCT CC-31
HuVk2aBACK	5'-GAT OTT GTG ATG ACT CAG TET CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACO ACA CTC ACG CAG TCT CC-3'
HuVk6sBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuV\1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
H¢V\2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
Huva3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVA4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuV\\5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVλδBACK	5'-AAT TIT ATG CTG ACT CAG CCC CA-3'

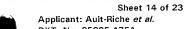
D. Human (1 kappa forward primers (anti-yense)

w. memana kap	ha los mara bismos samis across
HuJk IFOR	5'-ACG TIT GAT TTC CAC CTT GGT CCC-3'
Hwk2POR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJk3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJk4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HulkSEOR	5'- ACG TOT & AT CTC C AG TOG TOT CCC 3'

D. Human I lambda forward primers (anti-sense)

Hu JAIFOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
Hu Ja2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
Hu Ja4-5FOR	5'-ACC TAA AAC GOT GAG CTG GGT CCC-3'

continues



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AND HIGH THROUGHPUT SCREENING FIGURE 13B

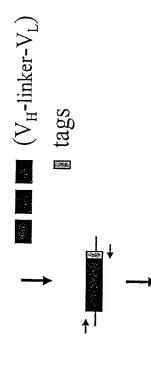
TABLE 3	Continu	
2. Linker	fraemen	PCR
		IH for scFv linker (sense)
		FR4 heavy -linker
		RHulHI-2 5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
		RHuJH3 5'-GG ACA ATG GTC ACC UTC TCT TCA GGT GG-3'
		RHuJH4-5 5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
		RHulh6 5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'
r.	5 D	Vk for sarv linker (anti-sense)
r.	. Meyerne	
		RHuVklaBACKEV 5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
		RHuVk2aBACKFv 5'-OG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
		RHOVE 3 BACKEY 5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
		RHuVk4aBACKFV 5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
		RHuVkSaBACKFy 5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'
		RHuVk6aBACKFv 5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'
F.	. Reverse	VA for scFv linker (anti-sense)
		FRI light linker
	RHuVA	RACK IEV 5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
		RACKOEV 5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
	$RHuV\lambda$	BACK3aFv 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
		BACK3bPv 5'-GG GTC CTG AGT CAG CTC AGA AGA CGATCC GCC ACC GCC AGA G-3'
		BACK4Fy 5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
		BACKSEV 5'-GA COG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3' BACK6EV 5'-TG GGG CTG AGT CAG CAT AAA ATT COA TCC GCC ACC GCC AGA G-3'
	RHuVλ	BACK6FV 5'-1'G GGG CTG AGT CAG CAT AAA ATT COA TCC GCC ACC GCC AGA G-3
3. Pull-thr	ough pr	imers for introduction of restriction sites"
C	i. Humar	VII buck (Sfi) primers (sense)
H	IuVHlaF	ACKS6 - FRI heavy
5	'-crc d	TC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
H	íuVH2a≢	BACKSfi
		TC GCA ACT GC <u>G GCC</u> CAG CC <u>G GCC</u> ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3' BACKSB
5	'-GTC C	TC GCA ACT GC <u>G GCC</u> CAG CC <u>G GCC</u> ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'
! -	łuVH4aŁ	BACKS6
5	r-GTC ¢	TO GOA ACT GOG GOO CAG COG GOO ATG GOO CAG GTG CAG CTG CAG GAG TOG GG-3'
ŀ	iuVH5a	ACKS6
	,	TO GOA ACT GOG GOO CAG COG GOO ATG GOO CAG GTG CAG CTG TTG CAG TOT GO-3'
F	TuVH6u	BACKSB TO GCA ACT GC <u>G GCC</u> CAG CC <u>G GCC</u> ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3′
2) -UIC 4	TO GCA ACT GCGGGC CAG CCGGGC ATO GCC CAG GTA CAG CTG CAG GAG TO TO
1.	1. Huma	ı I kappa forward (Not) primers (anti-sense)
	•	IuJk1FORNot FR4 light
		'-GAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACG TTT GAT TTC CAC CTT GGT CCC-3'
		Hulk2FORNot 5'-OAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACG TTT GAT CTC CAG CTT GGT CCC-3'
		H. Human J kappa jorward (Not) primers (anti-sense) (Continued) FR4 light
		HUJK3FORNOL FR4 light
		Hulk4FORNot
		5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
		HulkSFORNot
	;	5'-GAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACG TTT AAT CTC CAG TCG TGT CCC-3'
	į	H. Human J lambda forward (Not) primers (anti-sense)
		Hu J1FORNOT FR4 light
		S'-DAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
		Ho JI2-3FORNOT
		5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
		Un HA-SECORNICT

5'-GAO TCATTC TCG ACT TGC GGC CGC ACC TAA AAC GGT GAG CTG GGT CCC-3'

[&]quot;Recognition site for restriction enzyme is underlined."

step I

Tag and assemble immunoglobulin genes

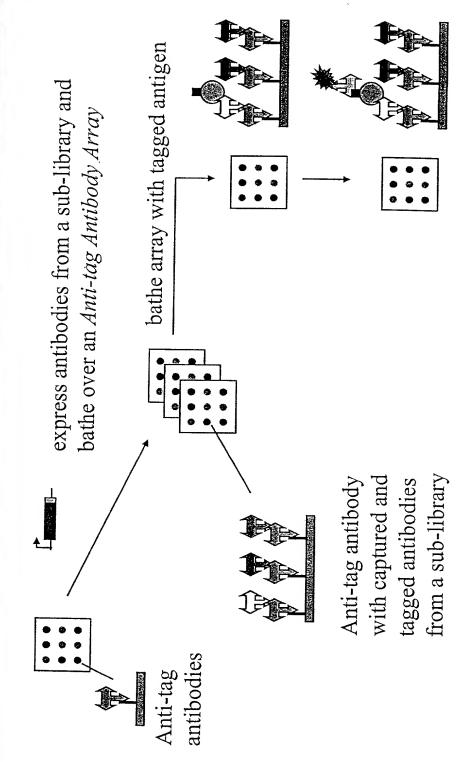


Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers



1,000 sub-libraries

AND HIGH THROUGHPUT SCREENING TAGS AND USES THEREOF FOR NESTED SORTING For: COLLECTIONS OF BINDING PROTEINS AND Priority claimed to 60/219,183 DKT. No. 25885-1751 Sheet 15 of 23



ID spot containing the antigen with a labeled developing Ab

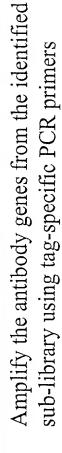
FIGURE 14B

AND HIGH THROUGHPUT SCREENING DAGS AND USES THEREOF FOR NESTED SORTING For: COLLECTIONS OF BINDING PROTEINS AND Priority claimed to 60/219,183 DKT. No. 25885-1751 Applicant: Ault-Riche et al. Sheet 16 of 23 A WHITE & MCAULIFFE LLP

<u>ынэ вэттэн</u>



step II



was 1,000,000,000 then each spot in this array If the starting diversity of the master library will have 1,000 different types of rAbs



Express and purify the antibodies



Re-distribute over an Anti-tag Antibody Array

was 1,000,000,000 then each spot in this array If the starting diversity of the master library will have a single type of rAb



Re-survey to ID the antibody of interest

FIGURE 14C

AND HIGH THROUGHPUT SCREENING TAGS AND USES THEREOF FOR NESTED SORTING FOI: COLLECTIONS OF BINDING PROTEINS AND DKT: No. 25885-1751 Applicant: Ault-Riche et Sheet 17 of 23

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summary

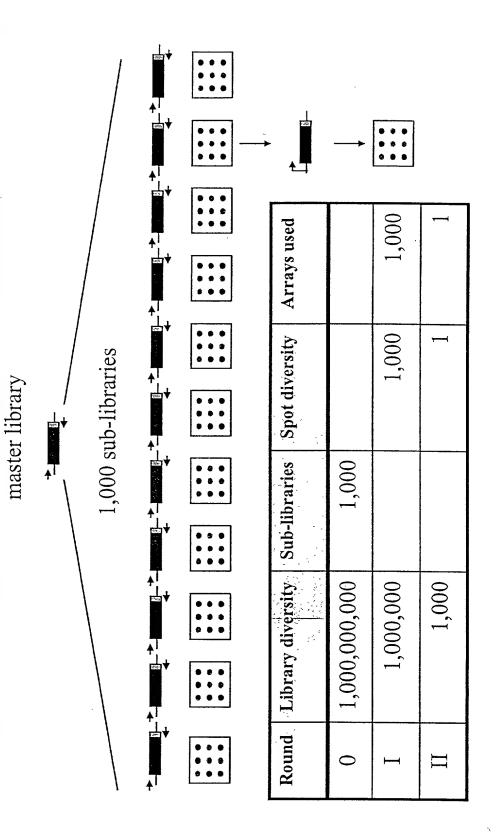


FIGURE 14D

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AND HIGH THROUGHPUT SCREENING

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- Modification searches

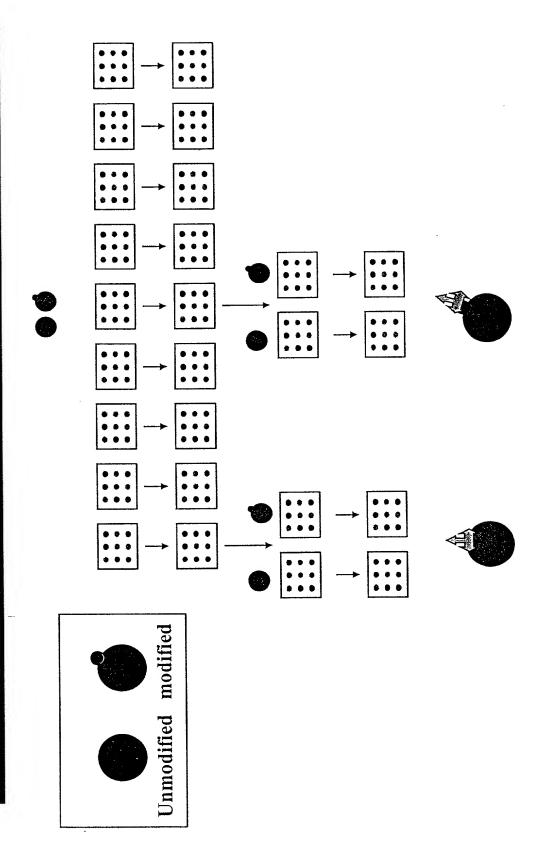


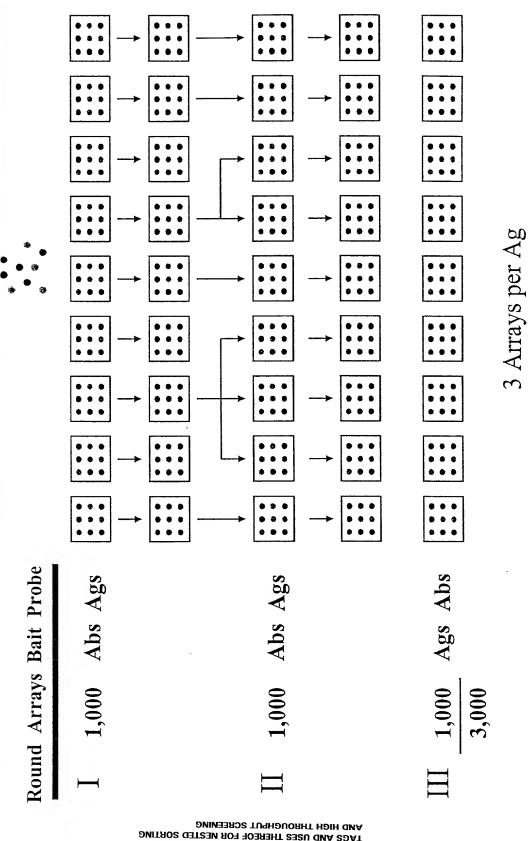
FIGURE 15

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For:COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING THEN USES THEREOF FOR NESTED SORTING THE COLLECTIONS OF BINDING PROTEINS AND USES THEREOF FOR NESTED SORTING THE COLLECTIONS OF STREET SORTING THE COLLECTIONS OF STREET SORTING THE COLLECTIONS OF STREET SORTING THE COLLECTION OF STREET SORTING THE STREET SORTING THE COLLECTION OF STREET SORTING THE S

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3



TAGS AND USES THEREOF FOR NESTED SORTING FOR: COLLECTIONS OF BINDING PROTEINS AND Priority claimed to 60/219,183 DKT. No. 25885-1751

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Enzyme engineering

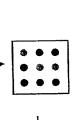
Mutated genes Error-prone PCR or Gene Shuffling Natural gene(s)

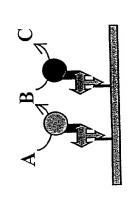
tag the gene to be mutated

• mutate genes and create sub-libraries

distribute mutants over arrays

• probe the arrays with labeled substrates





Spots can contain mixtures of enzymes for detection or pathway engineering

FIGURE 17

AND HIGH THROUGHPUT SCREENING TAGS AND USES THEREOF FOR NESTED SORTING FOR: COLLECTIONS OF BINDING PROTEINS AND E81,012/08 of beamists ythion9 DKT. No. 25885-1751 Applicant: Ault-Riche et al. Sheet 21 of 23 яты а этим ините в мелисияна инияна

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Protein interaction mapping

cDNA library

- human tissue
- pathogen

yeast

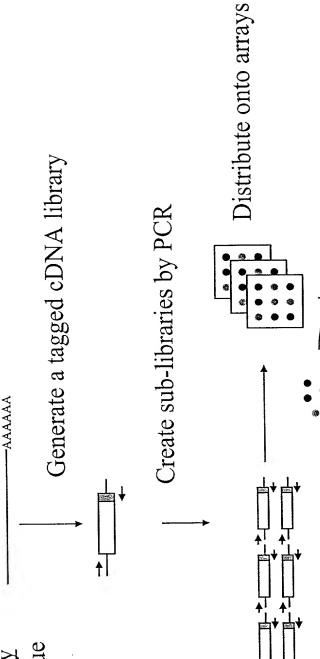


FIGURE 18

FOI: COLLECTIONS OF BEODING PROTEINS AND HIGH THROUGHPUT SCREENING

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неггев

probe arrays with one or several labeled proteins,

peptides, or drugs

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FIGURE 19

